THE COLORED CORPUSCLES OF THE BLOOD OF THE PURPLE SEA SPIDER, ANOPLODACTYLUS LENTUS WILSON

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In Europe, the cells of the circulating fluids of invertebrates have been given considerable attention. The data on the corresponding animals of America are much less complete. Of great interest, especially from an evolutionary viewpoint, are the cells of more or less fixed form in which the respiratory pigments, hemerythrin or hemoglobin, are enclosed. The other recognized respiratory proteins, chlorocruorin and hemocyanin, are reported as occurring only in solution in the blood or body fluids.

A survey of the literature yields little information on the blood cells of the pycnogonids. Cells of definite form, designated as "ballons," were described by Dohrn (1881) as occurring in the pycnogonids of the Gulf of Naples. These observations were supplemented by Cuénot (1891), who studied the pycnogonids in the vicinity of Banyuls. According to the latter author these cells contain an unidentified albuminoid which is either colorless or of a light neutral tint and does not change color on exposure to the air. The present report deals with the colored corpuscles of the sea spider, *Anoplodactylus lentus* Wilson (*Phoxichilidium maxillare* Stimpson), which is found in considerable numbers in the vicinity of Woods Hole.

The corpuscles of this animal range in color from light pink, deep pink, and lavender to purple and dark blue, and appear to be extremely specialized cells. The color of the blood, however, is blue.

Vital Observations on Entire Animals and Amputated Legs

The blood spaces of the body of pycnogonids are divided into dorsal and ventral halves by septal membranes which are not limited to the body but run to the tips of the legs. The course of the circulation is outward in the inferior or ventral sinus and inward towards the heart in the superior or dorsal sinus. In forms like the species studied, with small bodies and long, slender legs, the circulation within the appendages is dependent largely on limb movements and on the contractions of the intestinal cæca which extend into the appendages.

Anoplodactylus is sufficiently small and translucent that the entire animal may be compressed under a large coverslip and the heart-beat and circulation observed under relatively high magnification. More satisfactory observations, however, may be made on amputated legs mounted in sea water. The cut end is quickly plugged by a clot and practically no blood is lost. A high dry lens or even a 2 mm. oil immersion can be used on many of these preparations. The contractions of the intestinal cæca persist for a long time and the contents of the sinuses surge back and forth with each pulsation, permitting observations on the corpuscles as they change position and orientation.

The corpuscles are extremely thin discs, irregularly oval or lenticular in outline, tapering at the ends into slender processes of varying length (Figs. 1 to 6), and in optical section these cells are seen to possess thickened, rounded margins. The nucleus is occasionally centrally located (Fig. 2), but usually is found in the thickened margin about midway between the two poles (Fig. 7). In the thin central region the pigment is scanty, becoming denser toward the periphery of the cell. The terminal processes do not contain pigment and may be readily overlooked in fresh preparations. The cells vary widely in size. These variations may be due to the degree of maturity of the corpuscles since the small cells usually possess less color.

In the smaller elements the pigment is uniformly distributed throughout the body of the cell; in the larger, it is marked off into irregular areas by clear bands which tend to run longitudinally, although transverse bands are not uncommon. The nature of these divisions is not clear.

As already noted, there is a wide range in the color of the corpuscles when observed either in the intact animals or in amputated appendages. Some of the variations may be due to differences in concentration of the pigment but there is some evidence that the deep blue represents the completely oxidized form, while the lavender and pink shades are caused by varying degrees of reduction of the pigment.

The cells are extremely flexible and may be folded longitudinally, twisted spirally, or folded transversely as they are subjected to pressure from the pulsating intestinal cæca. Recovery from such deformation is rapid. The tips of the terminal processes are apparently adhesive in nature, and cells frequently become attached to the walls of the sinuses by one or both ends and sway and twist about as the blood surges back and forth.

Supravital Observations

Sufficient blood for supravital observations on the corpuscles may be obtained by gently pressing the amputated legs. The blood is fre-

quently mixed with ova and portions of the cæcal epithelium, but excellent preparations of almost pure blood may sometimes be obtained. The supravital preparations permit a more critical study of the morphology of the corpuscles, but degenerative changes occur more rapidly than when the blood is in the sinuses of the leg.

No formed bodies other than the nucleus can be distinguished in the fresh cells. In stale preparations the pigment loses its homogeneity and denser globules of varying size suspended in a paler fluid appear (Fig. 8). If the globules are large, no Brownian movement is discernible but smaller bodies may exhibit intense activity. The clear bands appear to form definite boundaries within the cell and the vibrating globules are unable to pass across them.

In sealed coverslip preparations which have stood for several hours many cells develop fine beaded processes on their surfaces. These processes contain pigment and usually show Brownian movement. Minute globules may separate from their free ends and persist for some time as free bodies in the plasma.

In hypertonic solutions the cells are shortened and thickened and irregular in outline (Fig. 10), resembling in some respects the crenated corpuscles of the vertebrates. In hypotonic solutions, produced by adding distilled water at the margin of the coverslip, the cells swell, lose their thin, flattened form (Figs. 11 and 12) and eventually become

EXPLANATION OF PLATE

All drawings are from fresh preparations and were outlined at the same magnification (× 1500) by means of a camera lucida. The nuclei appear as light areas.

Plate I

Explanation of Figures

1 to 6. Surface views of colored corpuscles of *Anoplodactylus lentus*, showing variations in size, form, and distribution of the pigment.

7. Optical section of a corpuscle viewed on edge drawn at the level of the marginal nucleus.

8. A corpuscle showing the formation of dark globular masses which frequently appear in sealed preparations which have stood for several hours.

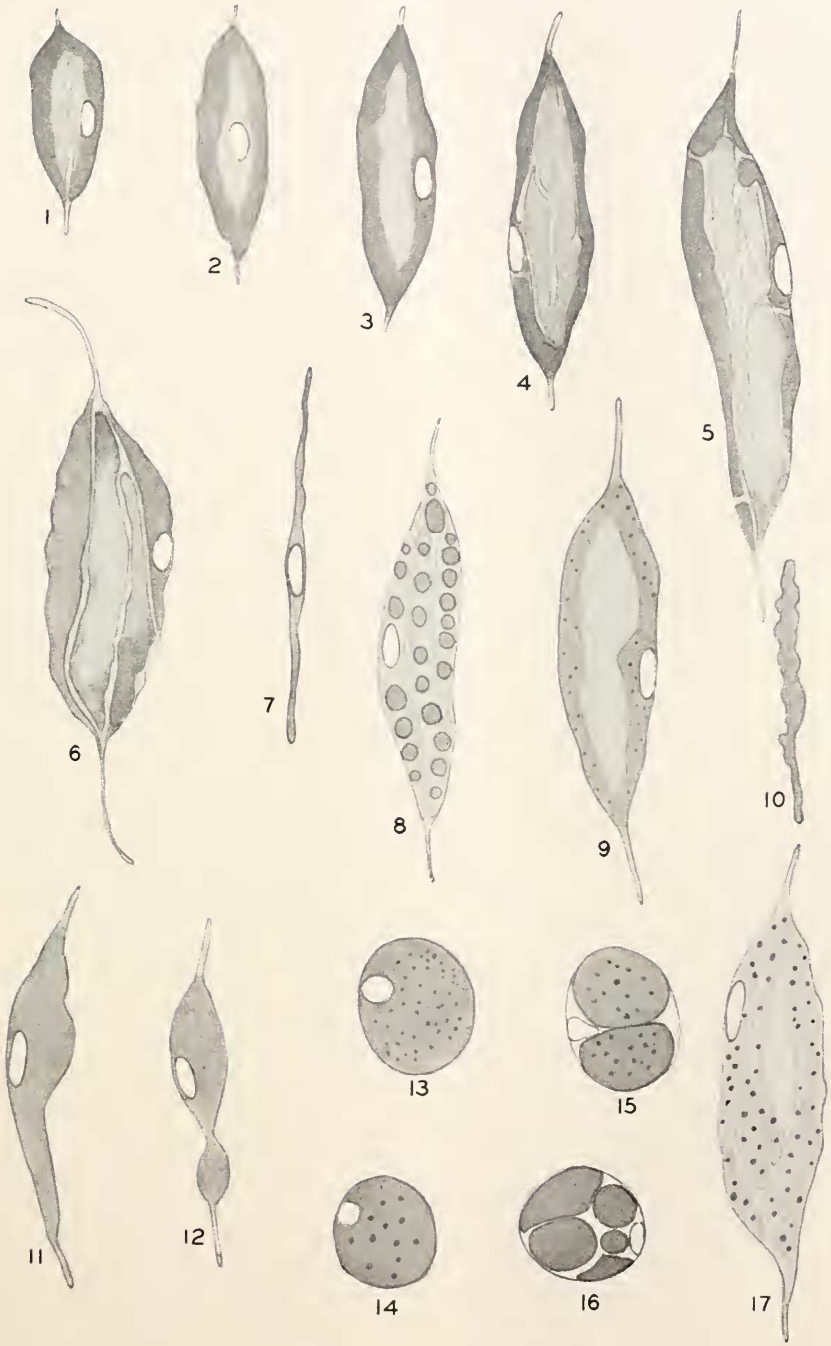
9. A corpuscle after supravital staining with neutral red and Janus green B. No characteristic neutral red bodies or mitochondria could be demonstrated. Dark, refractile granules of varying size, exhibiting active Brownian movements, appeared after the application of the dyes.

10. A corpuscle in a hypertonic solution viewed on edge showing a condition comparable to crenation in the vertebrate erythrocyte.

11 and 12. Early stages in the transformation of the thin flattened corpuscles into spheres.

13, 14, 15, and 16. Spherical corpuscles, usually containing induced granules of varying size, were produced by several methods. The occurrence of the pigmented content in separate masses is probably correlated with the presence of the clear septa present in many normal corpuscles.

17. Corpuscle after exposure to dilute hydrochloric acid, showing loss of pigment and the appearance of dense refractile granules.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converted into spheres (Figs. 13, 14, and 15). Such cells usually contained induced bodies of varying size which are in active Brownian motion. In these spherical cells (Figs. 15 and 16) the pigment is frequently divided into separate masses, a condition which is probably correlated with the presence of clear bands in the normal corpuscles.

Fresh preparations stained supravitally with neutral red, Janus green B, or brilliant cresyl blue reveal no additional cytoplasmic constituents such as mitochondria, neutral red bodies, or patterns of reticulation. In all cases, however, the large, more deeply pigmented cells contained densely colored bodies of varying size (Fig. 9), which exhibited different degrees of Brownian activity. This reaction to vital dyes appears comparable to that obtained in stale preparations, although the induced bodies are never so large. It is probably the result of injury. The characteristic color of any of the dyes was never obtained; it was masked by the natural pigment of the cell.

The Nature of the Intracellular Pigment

The color range of the corpuscles in entire animals and in amputated legs appeared the same. Attempts were made to determine the nature of the pigment and the factors producing the variations in color but the results were not at all conclusive. Only minute amounts of blood could be obtained by expressing the fluid from the legs so that all tests were carried out on the slide by drawing the various solutions under the coverslip.

It was obvious that the concentration of pigment varied in individual cells, probably directly with the degree of their maturity. The hydrogen ion concentration of the medium did not appear to influence the color of the pigment. Solutions of hydrochloric acid in sea water (pH 3.0) did not cause any change in color, but greater acidity caused a loss of pigment from the cell, probably due to cytolysis. Following the extraction of the pigment, numerous dense, refractile bodies were noted in the portions of the cell from which pigment was lost (Fig. 17). Solutions of sodium hydroxide (pH 9.0) also produced no color change but the cells rapidly assumed the spherical form (Fig. 17).

Reducing agents produced more positive results. Sodium hydrosulphite was applied in pulverized form at the margin of the coverslip and sufficient sea water added so that the solution might be drawn under the slip. Changes in color were noted after 15 minutes. Cells which were purple and deep blue changed to light brown and brownish yellow. Lavender cells became pink and pink cells colorless. After two hours the majority of the cells were colorless but a number of light yellow and pale pink cells could still be seen in the field. After four hours the

reduction of the color was almost complete. The reduction of the pigment was also accomplished by applying potassium cyanide in a similar manner. The more deeply colored cells in this instance faded to various shades of slate and gray before becoming colorless. Decolorization was frequently complete in about three quarters of an hour. Attempts to reoxidize by air the pigment in preparations treated with sodium hydrosulphite were unsuccessful, and questionable results were obtained with potassium ferricyanide. This may have been due to limitations of the method employed in handling such small amounts of blood.

Fresh preparations sealed with vaseline and kept for as long as forty-eight hours were not appreciably changed in color by the exclusion of air. Blood drawn from animals which were sealed in vials of boiled sea water until all activity had ceased and they had become limp seemed to contain a lower percentage of purple and blue cells than normal animals. The blood picture as regards color of the corpuscles, however, is very variable and comparisons are difficult. Macroscopically the blood seemed lighter in color, and when exposed to air seemed to grow darker.

Microspectroscopic examination of individual cells from dry unstained smears did not demonstrate any definite absorption bands for the pigment preserved in this manner. These preliminary tests are obviously inadequate to determine the identity or nature of the pigment. There is, however, some slight evidence that it may undergo reversible oxidation-reduction changes or play some rôle in oxygen transfer.

The variations in color of the corpuscles in the circulation appear to depend on two factors: the relative concentration of the pigment within the cells and the degree of its reduction or oxidation. A third possibility—the presence of another pigment, especially in the dark blue cells which do not decolorize in the same manner as the lighter cells—should not be overlooked.

One of the most widely distributed blue pigments in the animal kingdom is hemocyanin. This substance, however, has not been reported within cells of the circulating fluids but has always been found dissolved in the plasma. Another, as yet unidentified, blue-purple pigment has been reported as occurring in the blood and tissues of *Chromodoris zebra* and related species (Crozier, 1914, 1922) and some of the physical and chemical properties of this pigment have been described by Preisler (1930). Further studies on the intracorpuscular pigment of the blood of *Anoplodactylus* are needed in order that its chemical nature and functional significance may be ascertained.

I am indebted to Professor A. C. Redfield for helpful suggestions during this study.

Summary

The blood of the sea spider, *Anoplodactylus lentus,* contains numerous pigmented corpuscles. The cells are thin, flexible discs of lenticular or irregularly oval outline with a marginally located nucleus. The poles of the cells are elongated into slender processes which are adhesive.

The corpuscles range in color from pink, lavender, and purple to deep blue. The pigment may be homogeneously distributed or limited to areas marked off by clear bands.

These cells are readily deformed by changes in the concentration of the medium. Staining reactions characteristic of vertebrate erythrocytes are not produced by exposure supravitally to neutral red, Janus green B or brilliant cresyl blue.

Preliminary tests indicate that variations in the color of the pigment are not dependent on hydrogen ion concentration.

The pigment may be decolorized with potassium cyanide or sodium hydrosulphite, but attempts to restore the color were inconclusive. The small amounts of blood obtainable render tests of this nature difficult.

This pigment, obviously neither hemoglobin or hemerythrin, occurring intracellularly in the blood of an invertebrate, and possibly of respiratory significance, merits the attention of physiologists.

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